

Chapter 20

A Closer Look at Cyclodextrins in Mycotoxin Analysis

Michael Appell and Chris M. Maragos

Mycotoxin Research Unit, USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604

Cyclodextrins are a class of cyclic oligosaccharides with a variety of applications, including use as recognition components for low molecular weight molecules in methods of detection. These cycloamyloses are of special interest in mycotoxin analysis for enhancing spectroscopic properties of several mycotoxins under aqueous conditions, including aflatoxins, zearalenone, ochratoxin A, and through chemical derivatization, T-2 toxin. Spectroscopic studies and applications of cyclodextrins are frequently associated with inclusion complex formation. Theoretical modeling studies suggest cyclodextrin-mycotoxin interactions are influenced by the size and nature of the mycotoxin and favorable binding energies. The results provide a better understanding of the effectiveness and limitations of incorporating cyclodextrins into mycotoxin analysis.

Introduction

A broad range of approaches are applied to prevent and control natural mycotoxin contamination of agricultural commodities. The effectiveness of these approaches is determined by accurate analysis of toxin levels and validation of methods of detection (1-4). Concern over mycotoxin contamination levels has spurred development of new detection methods to improve accuracy, sensitivity, time, and selectivity. Several materials capable of improving mycotoxin analysis are based on selective recognition of analytes, and these materials have been successful in a variety of detection formats, including ELISAs, selective affinity columns (immunoaffinity), and test strips. In addition, inherent properties of the toxins, such as fluorescence, permit improvements in detection levels and selectivity. Less selective materials, including cyclodextrins, offer a general means to improve detection. The use of cyclodextrins in mycotoxin analysis has focused on spectroscopic phenomena, as well as size exclusion (5-7). This manuscript reviews recent developments in the use of cyclodextrins for mycotoxin analysis, and examines the parameters important for application of cyclodextrins in mycotoxin detection.

Cyclodextrins: General Overview

Cyclodextrins are cyclooligosaccharides consisting of six to eight glucopyranose units connected through α -(1,4) linkages (8). These cycloamyloses are synthesized enzymatically from degraded starch (7, 8). Cyclodextrins are of particular interest as "generic" receptors for the ability to form guest-host complexes with their cavities. Similarities between cyclodextrins with six (α -), seven (β -), and eight (γ -) glucopyranose subunits include a 4C_1 conformation, a face possessing all 6-hydroxymethyl substituents, and a large rim with secondary hydroxyl groups in the 2- and 3- positions. Noteworthy differences between α -, β -, and γ - cyclodextrins are solubility and cavity volume, with the larger cyclodextrins possessing larger cavities. It is of interest that higher concentrations of cyclodextrins can form more complex structures (9).

Properties of cyclodextrins can be tailored through chemical derivatization, however, this approach is complicated by similar reactivity of dozens of hydroxyl groups. Despite the lack of diverse functionality, moderate selectivity can be achieved by exploiting the difference in chemical reactivity attributed to the three positions of the hydroxyls in the glucopyranose subunit (10). The 6-hydroxymethyl substituents can be selectively modified by reactions limited to

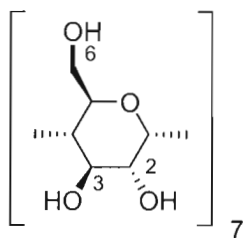
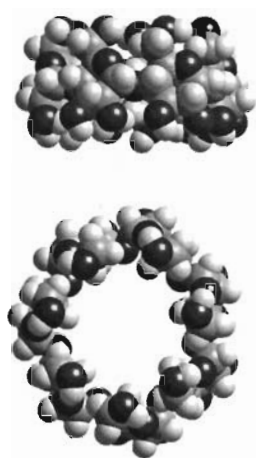
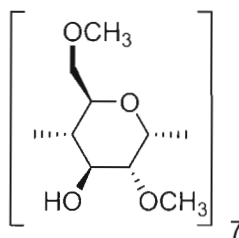
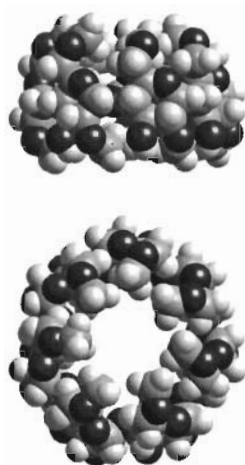
β -Cyclodextrin**DIMEB**

Figure 1. Structure of β -cyclodextrin and 2,6-dimethyl- β -cyclodextrin (DIMEB).

primary hydroxyls. Selectivity between the 2-hydroxyls and 3-hydroxyls can be achieved by the increased acidity of the 2-hydroxyls and the influence of the cooperative hydrogen bond network (10-12). Mixtures of cyclodextrin derivatives have found utility in analytical methods. One of the most popular derivatives is heptakis(2,6-di-*O*-methyl)- β -cyclodextrin, DIMEB, which occurs as a mixture of cyclodextrins substituted with methoxy groups in various 2- and 6- positions (see Figure 1). The methylation increases solubility compared to β -cyclodextrin, and is capable of greater enhancement of fluorescence intensity for several mycotoxins under certain conditions (5, 13).

Recent Applications of Cyclodextrins in Mycotoxin Analysis

Cyclodextrin-induced fluorescence enhancement of mycotoxins provides a general mechanism for selective detection by utilizing the hydrophobic cavities of cyclodextrins to overcome the fluorescence quenching properties of water (14). Applications of cyclodextrins in mycotoxin detection include recognition materials in chromatographic separations, additives to growth media to enhance

qualitative assessment of toxin presence, and modulators of quantitative analysis of mycotoxins. These types of mycotoxin-related uses have been reviewed (5-7); however, several recent developments are noteworthy.

Aflatoxin production by *Aspergillus* species can be monitored in growth media within the timeframe of three to ten days by cyclodextrin-assisted visual detection (15, 16). A fiber optic room temperature phosphorescence method has been developed for aflatoxin detection in growth media using β -cyclodextrin and the surfactant sodium deoxycholate (17). The fiber-optic based method reduced the time for detection of aflatoxins in culture medium to within 36 hours. Aflatoxin M₁ contamination of Slovenian milk and cheese has been detected using culture media supplemented with methyl- β -cyclodextrin (18, 19). In addition, a method has been developed to detect *Aspergillus* species from Iranian pistachios using PCR-based techniques coupled with methylated- β -cyclodextrin-assisted fluorescence detection in growth media (20).

Aflatoxin B₁ in wheat can be detected by use of second order standard addition methods on the cyclodextrin-assisted fluorescence spectra with minimal sample preparation (21). The effectiveness of cyclodextrins to improve the detection limits of aflatoxin M₁ in milk has been studied by a rapid screening method using a photomultiplier tube detector (22). The instrument is capable of detecting aflatoxin M₁ in milk at 50 ppt without cyclodextrins, and inclusion of cyclodextrins enhanced the detection levels by ~50%. Fundamental studies probing the induced fluorescence enhancement properties of several surfactants, β -cyclodextrin, and calixresorcinarenes have been carried out on aflatoxins B₁, B₂, G₁, and G₂ (23).

The inclusion phenomenon of mycotoxin-cyclodextrin complexes has been investigated using computational modeling methods (24). Study of the fluorescence enhancement of aflatoxin B₁ by spectroscopy and molecular modeling identified a 1:1 relationship for the guest:host complex (25). Interactions of β - and γ - cyclodextrins with aflatoxin B₁, zearalenone, and ochratoxin A were investigated by an analysis developed for hydrophobic interactions of proteins and protein-ligand interactions (26, 27). Quenching effects of water on aflatoxin B₁ fluorescence were studied using time dependent density functional methods (28).

It is clear that the binding interactions of cyclodextrins are more complicated than simple stoichiometric relationships and detection-enhancing phenomena are influenced by the structures of toxin and cyclodextrin. A closer look at the interactions of zearalenone using both fluorescence spectroscopy and molecular modeling can shed light on the merits and limitations of the use of cyclodextrins in mycotoxin analysis. Herein, we report fluorescence spectroscopic studies of the interaction of zearalenone with popular cyclodextrins β -cyclodextrin and DIMEB. Furthermore, to gain insight into the interactions of cyclodextrins and mycotoxins, semi-empirical studies were carried out on complexes of cyclodextrins with zearalenone, zearalenone analog resorcylic acid, aflatoxin B₁, ochratoxin A, and T-2 toxin. The structures for zearalenone **1** and aflatoxin B₁ **2** are shown in Figure 2. The resorcylic acid **3** and coumarin **4** moieties are associated with the fluorescence of these molecules. Fluorescence of ochratoxin A **5** (see Figure 3) is related to the

dihydroisocoumarin **7** moiety, and detection of T-2 toxin **6** by fluorescence is possible through chemical derivatization with pyrene 1-carbonyl cyanide, **8**.

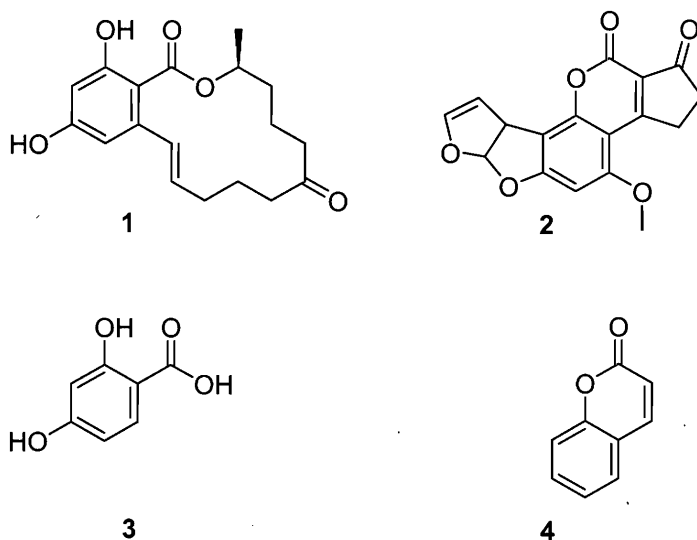


Figure 2. Structures for zearalenone (1), aflatoxin B₁ (2), resorcylic acid (3), and coumarin (4).

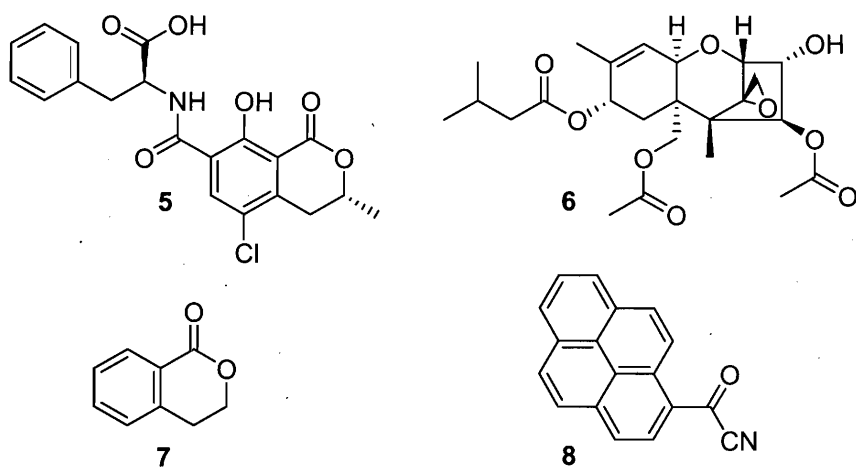


Figure 3. Structures for ochratoxin A (5), T-2 toxin (6), dihydroisocoumarin (7), and pyrene 1-carbonyl cyanide (8).

Materials and Methods

All chemicals and reagents were purchased from Sigma-Aldrich Company (St. Louis, MO, U.S.A.). Heptakis(2,6-di-*O*-methyl)- β -cyclodextrin (DIMEB) was used as the mixture provided. Deionized water was used for the preparation of all reagents (Nanopure II, Sybron/Barnstead). All solvents were HPLC grade.

Fluorescence Spectroscopy Fluorescence spectra were recorded on a Varian Cary Eclipse (Palo Alto, CA, U.S.A.) instrument in a 10 x 10 mm quartz cell. The zeaxalenone fluorescence intensity was measured at 460 nm after excitation at 270 nm. All experiments were recorded using a 5 nm slit width. Solutions were incubated for five minutes prior to analysis. Experiments were carried out at room temperature 22-26° C.

Molecular Modeling Calculations were carried out using Parallel Quantum Solutions (Fayetteville, AR, U.S.A.) hardware and software v3.2 (29). Initial structures were built using the HyperChem 7.52 program (Gainesville, FL, U.S.A.) and PM3 semi-empirical method (30). Geometry optimization was performed on delocalized internal coordinates using the Eigenvector Following Algorithm with the convergence criteria set at 1×10^{-6} Hartree and a gradient of less than 3×10^{-4} a.u. Carbon atoms are displayed in grey, oxygen atoms in black, and hydrogen atoms in white.

Data Analysis Data were analyzed using OriginPro v7.5 SR6 Software OriginLab Corporation (Northampton, MA, U.S.A.). Experimental results were fitted with linear and sigmoidal regression functions.

Results and Discussion

Influence of Cyclodextrins on Fluorescence of Zeaxalenone

The effect of various cyclodextrins on the fluorescence intensity of zeaxalenone (10 μ M) over pH values of 4 to 10 (100 mM sodium phosphate buffer) is depicted in Figure 4 for α -, β -, γ -cyclodextrins and DIMEB. It is noteworthy that α -cyclodextrin, with its smaller cavity, does not significantly enhance zeaxalenone fluorescence, and β -cyclodextrin exhibits the greatest enhancement of the unsubstituted cyclodextrins. DIMEB has the most significant fluorescence enhancement at the concentration studied (10 μ M

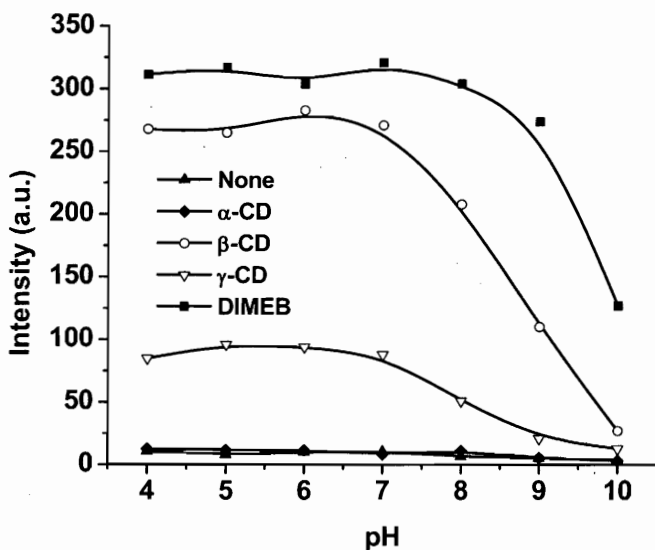


Figure 4. Effect of pH on fluorescence enhancement of zearalenone by cyclodextrins (100 mM sodium phosphate).

zearalenone, 1 mM cyclodextrin). Zearalenone fluorescence is quenched at higher pH. Starting at pH 8, fluorescence intensity is decreased for zearalenone in the presence of all cyclodextrins studied.

The influence of β -cyclodextrin and DIMEB concentration (0.01 mM and 1 mM) on the corrected fluorescence ($F-F_0$) of zearalenone is displayed in Figure 5. The fluorescence intensity of 10 μ M zearalenone is dependent on cyclodextrin structure and concentration for concentrations up to 0.5 mM. However, at cyclodextrin concentrations higher than 1 mM, β -cyclodextrin and DIMEB exhibit similar fluorescence-enhancing properties for 10 μ M zearalenone. The implications of this dependence of fluorescence intensity enhancement on cyclodextrin concentrations should be an important consideration in the analysis of structure-activity relationships involving zearalenone and cyclodextrins.

Frequently, cyclodextrin structure-activity relationships are characterized as ratios of normalized emission intensities of an analyte at a single cyclodextrin concentration. The normalized emission intensities of zearalenone for several concentrations of cyclodextrins in sodium phosphate buffer (100 mM, pH 7.0) are shown in Table 1. For an equal concentration of cyclodextrin and zearalenone (10 μ M), the ratio for normalized emission intensity is 3.6. However, as the concentration of cyclodextrin increases, the effect of the structure-activity relationship decreases for a comparison of DIMEB to β -CD.

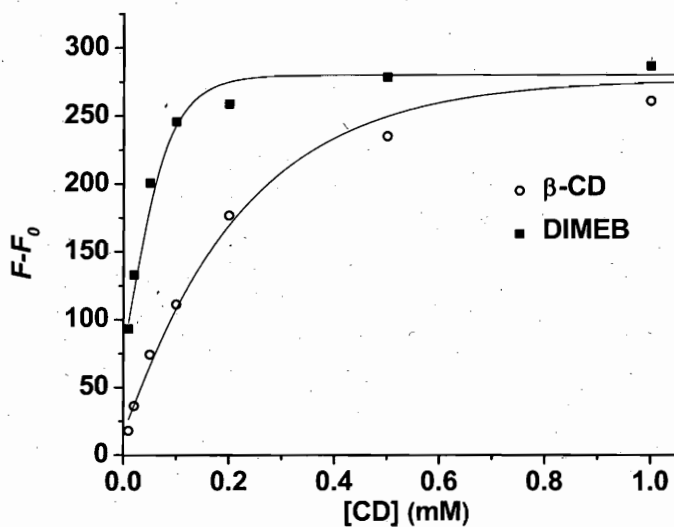


Figure 5. Influence of [CD] on fluorescence enhancement of zearalenone (10 μ M zearalenone, pH 7.0).

Table 1. Dependence of normalized emission of 10 μ M zearalenone at various concentrations of cyclodextrins.

[Cyclodextrin] (mM)	F/F_0 Zearalenone		
	β -CD	DIMEB	DIMEB/ β -CD
0.01	2.7	9.6	3.6
0.05	7.9	19.6	2.5
0.10	11.7	23.8	2.0
0.5	22.8	26.8	1.2
1.0	23.8	27.5	1.2
3.0	27.9	27.9	1.0

The spectrofluorimetric studies of the interaction of cyclodextrin with zearalenone can be analyzed by Scatchard plot analysis (see Figure 6):

$$\frac{(F - F_0)}{[CD]} = (F_\infty - F_0)K - (F - F_0)K$$

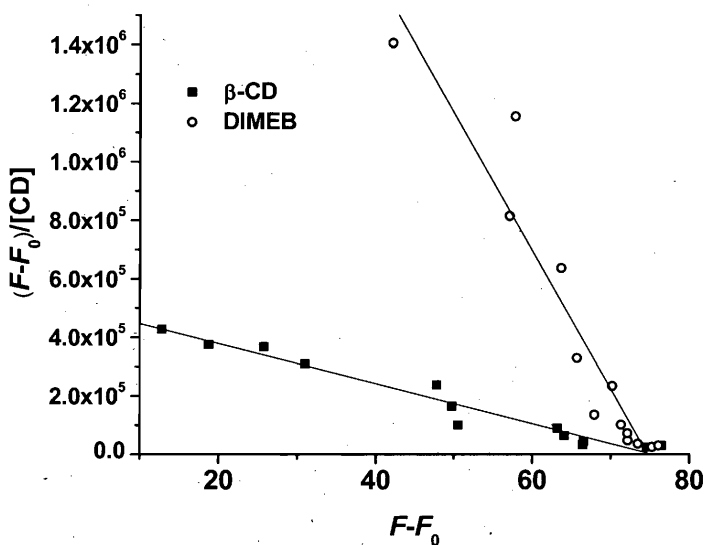


Figure 6. Scatchard plot of $(F-F_0)/[CD]$ vs. $F-F_0$ for zearalenone in the presence of β -cyclodextrin and DIMEB ($3 \mu\text{M}$ zearalenone).

Where F is the fluorescence intensity of the zearalenone:cyclodextrin complex, F_0 is the fluorescence intensity of free zearalenone, F_∞ is the fluorescence intensity when all zearalenone is complexed with the cyclodextrin, and K is the association constant (31-33). Scatchard plot analysis is a popular method to elucidate binding interactions and applicable to a broad range of binding phenomena. Furthermore scatchard plot analysis provides similar results to the Benesi-Hildebrand analysis commonly applied to study cyclodextrin-guest inclusion complexes (31, 32). The linearity of the $(F-F_0)/[CD]$ vs. $F-F_0$ plot indicates zearalenone forms a 1:1 complex with β -CD and DIMEB. Solving the equation gives a binding constant of $6,800 \text{ M}^{-1}$ for β -CD and $47,100 \text{ M}^{-1}$ for DIMEB.

Molecular Modeling Studies

Energetics and structural information of mycotoxin:cyclodextrin complexes were carried out using the PM3 semi-empirical method. It should be noted that PM3 calculations are limited in accuracy for certain types of hydrogen bond interactions compared to computationally expensive *ab initio* calculations (34). However, PM3 methods have been effective in the study of supramolecular inclusion complexes (35-37). Calculated parameters for the mycotoxin-cyclodextrin interactions are given in Table 2. The interaction energies associated with the heat of formation are calculated by:

$$\Delta E = E_{\text{Complex}} - (E_{\text{Mycotoxin}} + E_{\text{Cyclodextrin}})$$

Where $E_{Complex}$ is the heat of formation energy of the mycotoxin:cyclodextrin complex, $E_{Mycotoxin}$ and $E_{Cyclodextrin}$ are the energies for the free molecules, and ΔE is the stabilization energy. The value μ is the dipole moment of the mycotoxin-cyclodextrin bound complex.

Results presented in Table 2 offer some interesting insights into mycotoxin-cyclodextrin interactions. First, complex formation is driven by negative changes in energies for the mycotoxins investigated. Secondly, considerable variation exists in dipole moments, μ , suggesting that the mycotoxin has significant influence over the electronic effects of the bound complexes. Lastly, comparing ΔE of β -CD to DIMEB complexes of zearalenone and aflatoxin B₁, methylation lowers the energies of interactions of complexes suggesting the van der Waals interactions between DIMEB and the mycotoxin are important binding interactions.

Several mycotoxins are capable of forming multiple complexes with β -CD. Two orientations were identified for the zearalenone:cyclodextrin inclusion complex. The most favorable complex for zearalenone and the fluorescent derivative of T-2 toxin is with the fluorophore outside of the cavity, suggesting that binding interactions are more complex than exclusive fluorophore:cyclodextrin complexation. Through fluorescence spectroscopic studies it has been determined that the T-2 toxin pyrene derivative forms a complex with two cyclodextrins (5).

Table 2. Calculated parameters of mycotoxin-cyclodextrin interactions

Cyclodextrin Complex	Inclusion Moiety	ΔE (kcal/mol)	μ (Debye)
<i>Zearalenone</i>			
ZEN: β -CD	resorcylic acid	-8.34	2.48
ZEN: β -CD	ketone	-19.44	2.67
ZEN:DIMEB	resorcylic acid	-11.82	2.95
<i>Resorcylic acid</i>			
RA: β -CD	resorcylic acid	-5.71	2.02
RA:DIMEB	resorcylic acid	-7.85	2.84
<i>Aflatoxin B₁</i>			
AFB ₁ : β -CD	coumarin	-12.77	3.12
AFB ₁ : β -CD	dihydrofuran	-8.97	4.06
AFB ₁ : DIMEB	coumarin	-14.84	3.42
<i>T2-pyr</i>			
T2-pyr:DIMEB	T2-toxin	-30.25	3.37
T2-pyr:DIMEB	pyrene	-20.87	2.48
<i>Ochratoxin A</i>			
OTA: β -CD	phenyl	-8.91	4.39

Conclusion

The inclusion complexes of zearalenone and β -CD and DIMEB have been investigated by fluorescence spectroscopy and molecular modeling. Computational results were compared to cyclodextrin complexes of aflatoxin B₁, a pyrene derivative of T-2 toxin, and ochratoxin A. Structure-activity relationships for fluorescence spectroscopic studies of zearalenone with cyclodextrins are dependent on the type of cyclodextrin and pH. Theoretical calculations suggest that mycotoxins can interact with cyclodextrins in multiple binding modes and stabilization of the mycotoxin:cyclodextrin complex is associated with favorable interactions between the toxin and cyclodextrin. Other types of binding forces are likely to contribute to complex formation, including hydrophobic forces, conformational strain relief, and entropically favorable release of water from the cyclodextrin cavity (24). These results provide insight into the interaction of mycotoxins and cyclodextrins, and may assist in further applications of cyclomaltoses in methods of detection.

Acknowledgments

The authors thank Lijuan C. Wang for excellent technical assistance. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

1. Council for Agricultural Science and Technology (CAST). Mycotoxins: Risks in Plant, Animal and Human Systems. Task Force Report No. 139, CAST, Ames, Iowa, 2003.
2. Maragos C. M. Emerging technologies for mycotoxin detection. *J. Toxicol. Toxin. Rev.* **2004**, 23, 317-344.
3. Zheng, M. Z.; Richard, J. L.; Binder, J. A review of rapid methods for the analysis of mycotoxins. *Mycopathologia* **2006**, 161, 261-273.
4. Ueno, A. Review: Fluorescent cyclodextrins for molecule sensing. *Supramol. Sci.* **1996**, 3, 31-36.
5. Maragos, C. M.; Appell, M.; Lippolis, A.; Visconti, A.; Catucci, L.; Pascale, M. Use of cyclodextrins as modifiers of fluorescence in the detection of mycotoxins. *Food Addit. Contam.* **2008**, 25, 164-171.
6. Galaverna G.; Dall'Asta C.; Corradini, R.; Dossena, A.; Marchelli, R. Cyclodextrins as selectors for mycotoxin recognition. *World Mycotoxin J.* **2008**, 4, 397-406.
7. Marten Del Valle, E. M. Cyclodextrins and their uses: a review. *Process Biochem.* **2004**, 39, 1033-1046.
8. Szejtli, J. Introduction and general overview of cyclodextrin chemistry. *Chem. Rev.* **1998**, 98, 1743-1753.

9. Saenger, W.; Jacob, J.; Gessler, K.; Steiner, T.; Hoffmann, D.; Sanbe, H.; Koizumi, K.; Smith, S. M.; Takaha, T. Structures of the common cyclodextrins and their larger analogues - beyond the doughnut. *Chem. Rev.* **1998**, *98*, 1787-1802.
10. Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. Methods for selective modifications of cyclodextrins. *Chem. Rev.* **1998**, *98*, 1977-1996.
11. Rong, D.; D'Souza, V. T. A convenient method for functionalization of the 2-position of cyclodextrins. *Tetrahedron Lett.* **1990**, *31*, 4275-4278.
12. Takahashi, K.; Hattori, K.; Toda, F. Monotosylated α - and β -cyclodextrins prepared in an alkaline aqueous solution. *Tetrahedron Lett.* **1984**, *25*, 3331-3334.
13. Maragos, C. M.; Appell, M. Capillary electrophoresis of the mycotoxin zearalenone using cyclodextrin-enhanced fluorescence. *J. Chromatogr. A* **2007**, *1143*, 252-257.
14. Mallick, A. Purkayastha, P.; Chattopadhyay, N. Photoprocess of excited molecules in confined liquid environments: An overview. *J. Photochem. Photobiol., C* **2007**, *8* 109-127.
15. Rojas-Durán, T. R.; Fenta, C. A.; Vázquez, B. I. Franco, C. M. Sanz-Medel, A. Cepeda, A. Study of a room temperature phosphorescence phenomenon to allow the detection of aflatoxigenic strains in culture media. *Int. J. Food Microbiol.* **2007**, *115*, 149-158.
16. Abbas, H. K.; Zablotowicz, R. M.; Weaver, M. A.; Horn, B. W.; Xie, W.; Shier, W. T. Comparison of cultural and analytical methods for determination of aflatoxin production by Mississippi Delta *Aspergillus* isolates. *Can. J. Microbiol.* **2004**, *50*, 193-199.
17. Rojas-Durán, Sánchez-Barragán, I.; Costa-Fernández, J. M.; San-Medel, A. Direct and rapid discrimination of aflatoxigenic strains based on fiber-optic room temperature phosphorescence detection. *Analyst* **2007**, *132*, 307-313.
18. Torkar, K. G. Vengušt, A. The presence of yeasts, moulds and aflatoxin M₁ in raw milk and cheese in Slovenia. *Food Control* **2008**, *19*, 570-577.
19. Torkar, K. G. Vengušt, A. The microbiological quality of raw milk after introducing the two day's milk collecting system. *Acta Agriculturae. Slov.* **2008**, *92*, 61-74.
20. Rahimi, P.; Sharifnabi, B.; Bahar, M. Detection of aflatoxin in *Aspergillus* species isolated from pistachio in Iran. *J. Phytopathol.* **2008**, *156*, 15-20.
21. Hashemi, J.; Kram, G. A.; Alizadeh, N. Enhanced spectrofluorimetric determination of aflatoxin B₁ in wheat by second-order standard addition method. *Talanta* **2008**, *75*, 1075-1081.
22. Cucci, C. Mignani, A. G.; Dall'Asta, C.; Pela, R.; Dossena, A. A portable fluorometer for the rapid screening of M1 aflatoxin. *Sens. Actuators, B* **2007**, *126*, 467-472.
23. Goryacheva, I. Y.; Rusanova, T. Y.; Pankin, K. E. Fluorescent properties of aflatoxins in organized media based on surfactants, cyclodextrins, and calixresorcinarenes. *J. Anal. Chem.* **2008**, *63*, 751-755.
24. Lipowitz, K. B. Applications of computational chemistry to the study of cyclodextrins. *Chem. Rev.* **1998**, *98*, 1829-1873.

25. Aghamohammadi, M.; Alizadeh, N. Fluorescence enhancement of the aflatoxin B₁ by forming inclusion complexes with some cyclodextrins and molecular modeling study. *J. Lumin.* **2007**, *127*, 575-582.
26. Amadasi, A.; Dall'Asta, C.; Ingletto, G.; Pela, R.; Marchelli, R.; Cozzini, P. Explaining cyclodextrin-mycotoxin interactions using a 'natural' force field. *Bioorgan. Med. Chem.* **2007**, *15*, 4585-4594.
27. Cozzini, P.; Ingletto, G.; Singh, R.; Dall'Asta, C. Mycotoxin detection plays "cops and robbers": Cyclodextrin chemosensors as specialized police. *Int. J. Mol. Sci.* **2008**, *9*, 2474-2494.
28. Rameriz-Galicia, G.; Garduno-Jaurez, R.; Vargas, M. G. Effect of water molecules on the fluorescence enhancement of aflatoxin B₁ mediated by aflatoxin B₁: β -cyclodextrin complexes. A theoretical study. *Photochem. Photobiol. Sci.* **2007**, *6*, 110-118.
29. PQS version 3.2, Parallel Quantum Solutions, 2013 Green Acres Road, Fayetteville, AR 72703 USA
30. Hyperchem, Release 7.52, Hypercube Inc., 1115 NW 4th Street, Gainesville, FL 32601 USA
31. Berzas, J. J.; Alañón, A.; Lázaro, J. A. Cyclodextrin enhanced spectrofluorimetric determination of fluoxetine in pharmaceuticals and biological fluids *Talanta* **2002**, *58*, 301-309.
32. Verrone, R.; Catucci, L.; Cosma, P.; Fini, P.; Agostiano, A.; Lippolis, V.; Pascale, M. Effect of β -cyclodextrin on spectroscopic properties of ochratoxin A in aqueous solution. *J. Incl. Phenom. Macrocycl. Chem.* **2007**, *57*, 475-479.
33. Durán-Merás, I.; Muñoz de la Peña, A.; Salinas López, F.; Rodríguez Cáceres, M. I. Complexation study and spectrofluorimetric determination of pipemidic acid with γ -cyclodextrin. *J. Incl. Phenom. Macrocycl. Chem.* **2007**, *51*, 137-142..
34. Dolenc, J.; Koller, J. An improved semiempirical MO PM3 method for hydrogen bond systems. *Acta. Chim. Slov.* **2006**, *53*, 229-237.
35. Castro, R.; Berardi, M. J.; Cordova, E.; do Olza, M. O.; Kaifer, A. E.; Evanseck, J. D. Unexpected roles of guest polarizability and maximum hardness, and of host solvation in supramolecular inclusion complexes: A dual theoretical and experimental study. *J. Am. Chem. Soc.* **1996**, *118*, 10257-10268.
36. Song, L. X.; Wang, H. M.; Guo, X. Q.; Bai, L. A comparative study on the binding behaviors of β -cyclodextrin and its two derivatives to four fanlike organic guests. *J. Org. Chem.* **2008**, *73*, 8305-8316.
37. Leclercq, L.; Schmitzer, A. R. Multiple equilibria in the complexation of dibenzylimidazolium bromide salts by cyclodextrins: Toward controlled self assembly. *J. Phys. Chem. B* **2008**, *112*, 11064-11070.